

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : 10/777470 Confirmation No.: 2234  
Applicant : Abraham Phillip Lee  
Filing Date : 2/11/2004  
Title : MICROFLUIDIC DEVICES FOR CONTROLLED VISCOUS  
SHEARING AND FORMATION OF AMPHIPHILIC VESICLES  
Group Art Unit : 1709  
Examiner : Christine T. Mui  
Docket No. : 703538.4033  
Customer No. : 34313

## DECLARATION UNDER 37 C.F.R. § 1.131

Commissioner for Patents  
Mail Stop Box 1450  
Alexandria, VA 22513-1450

Sir:

We, Abraham P. Lee and Yung-Chieh Tan, declare as follows:

1. We are the only joint inventors of the above-identified patent application.
2. Before January 1, 2002, we conceived, in the United States, the invention disclosed and claimed in the above-identified patent application, and exercised reasonable diligence from conception until February 11, 2003, the filing date of U.S. Provisional Patent Application Serial No. 60/446798 to which the above-identified patent application claims priority under 35 U.S. § 120.
3. Exhibit 1 is a true copy of disclosure materials, from which the dates have been redacted, which record work performed before June 9, 2002, in the United States.

4. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereunder.

Dated: \_\_\_\_\_

\_\_\_\_\_  
Abraham P. Lee

Dated: 10/01/08

\_\_\_\_\_  
*Yung-Chieh Tan*  
Yung-Chieh Tan

OHS West:260523913.1  
703538-4033 BSL/BSL

703538.4033

Patent

4. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereunder.

Dated: Oct. 2, 2008  
Abraham P. Lee

Dated: \_\_\_\_\_

\_\_\_\_\_  
Yung-Chieh TanOHS West:260523913.1  
703538-4033 BSL/BSL

# EXHIBIT 1

## CONFIDENTIAL DISCLOSURE AND RECORD OF INVENTION FORM

Note: When completed, the *Disclosure and Record of Invention Form* is an important legal document. Care should be taken in its preparation. Please refer to accompanying instructions. If you desire assistance, please call the Office of Technology Alliances at (949) 824-7295. Information contained in this document is maintained in confidence by the University and normally will not be released to others (except with attorney client privilege, to research sponsors as required by contract, under appropriate secrecy agreements) until a patent application is issued, the information is published, a determination not to file a patent application is made, or as may be required by law. The information contained herein should not be disclosed to others outside the University, except as described in Section 8, without the approval of the University Office of Technology Alliances. It is *not* the practice of the University to send your Record of Invention to other University employees for peer review.

### ALL QUESTIONS MUST BE ANSWERED

1. **General Subject Matter:**

Microfluidic devices for controlled viscous shearing of oil-water emulsions of micro- and nano-scale droplets and the subsequent formation of amphiphilic vesicles (liposomes, polymer vesicles, micelles, etc.). The invention includes various microfluidic device designs for different nanomanufacturing features (speed of droplet formation, droplet size ranges, multi-lamellar amphiphilic layers, asymmetric vesicles). Also included are designs for post-assembly and post-processing of the droplets (splitting, fusing, sorting) and polymer emulsions. The combined features and functions of these devices will enable the integrated amphiphilic vesicle production-line, all on a single microfluidic chip. These amphiphilic vesicles are artificial cells with applications in drug delivery (including biomolecular nanomedicine such as DNA, peptides, proteins), combinatorial chemistry, nanoscale chemical reaction chambers, biomolecular devices (power, optical, electrical), and various biosensors.

2. **Names and Status (e.g. faculty, postdoc, graduate student) of persons connected with the work:**

In the event that a patent application is filed by the University, actual inventorship will be determined as a matter of law by a patent attorney.

Abraham Phillip Lee, Faculty  
 Yung-Chieh Tan, graduate student

3. **a) Brief description of the invention:**

What is it? How is it done? What is the purpose? What is the fundamental principle?

This first part of the invention includes various new microfluidic device designs for oil-water co-flows with tunable viscous shear forces higher than the immiscible interfacial tension forces to generate favorable conditions for droplet formation. The higher the shear force the smaller the droplet size. There are two major inventions in the droplet formation step: 1. the design of oil-water-oil sheath flow to establish stable interfaces with controllable shear forces. This enables reproducible, and batch fabrication of the devices. 2. The pressure gradient design to generate favorable conditions for high speed droplet formation. The second part of the invention is the method to generate bilayer membranes for artificial cells in microfluidic devices. The third part of the invention includes various microfluidic post-assembly and post-processing, that can further split droplets into even smaller droplets, fuse droplets into larger ones, or sort the droplets based on density and size.

This concept is depicted in its most general form in Fig. 1 and Fig. 2. Figure 1 illustrates the bilayer amphiphilic vesicles formation. First, a sheath flow of oil-water-oil is formed at the junction of 3 microchannels. The relative flow rates are controlled and dictate the viscous forces at the oil-water interfaces. This determines whether droplet formation is favorable and if it is, what sizes they form at. Second, by dissolving amphiphiles in the oil solutions a monolayer membrane is formed around the droplets to form monolayer vesicles. This vesicle is then delivered across the oil-water interface and forms a bilayer vesicle upon entering the aqueous solution. Figure 2 illustrates the droplet post-assembly and post-processing components in one integrated design. Droplets enter bifurcation of the microfluidic channels and are pressurized into both microchannels at this junction. The two pressures stretch the droplets and the corner of the channel walls assist in shearing apart the droplets. For fusing, larger droplets are entering a bifurcation joint from the right hand side and get trapped due to smaller size of the neck of the vertical channel. Smaller droplets are entering from the left hand side and are able to enter the vertical channel. In the process, the large size droplets experience an oscillating or pulsatile pressure that causes it to rock back and forth. This rocking motion "hammers" two droplets together to form a droplet double the original size. For the sorter, droplets passing from left to right are sorted by velocity. Again, at the bifurcation, there are two channels with different pressure drops to "attract" the droplets. The higher the velocity, the more likely the droplet is able to cross-over to the horizontal channel. Since the smaller droplets have higher velocity they will be collected in the horizontal channel and the larger ones will drop into the vertical channel (downwards).

The purpose of the invention is to generate artificial cell-like vesicles with tailored composition and size for various medical, biotech, materials development and sensing applications.

- b) The invention is a new:      ☒--Product      ☒--Process
- ☐--Composition      ☒--Method of use

(check all applicable)

4. **Funding source(s):**

List the funding source(s) for the project under which this invention was made. If applicable, identify by contract or grant number and name the Principal Investigator / Supervisor of each.

Funding Source / Sponsor	Contract or Grant Number	Principal Investigator / Supervisor
UCI startup funds		

5. **Proprietary materials:**

If any proprietary material (e.g., cell line, antibody, plasmid, computer software, or chemical compound) obtained from outside your laboratory was used to develop this invention, please check the box below or attach a copy of that agreement.

This invention utilized Data or Materials from:

- ☐--A subscription to the proprietary database Celera
- ☐--Affymetrix Chips
- ☐--Material Transfer Agreement (MTA)
- ☐--Other proprietary material (Please Explain)

(check all applicable)

6. **Relevant Dates:**

Item	Conception and First Written Description	First Successful Operation
Date:		
By whom	Abe Lee	Yung-Chieh Tan and Abe Lee
Where Recorded	Arlington, Virginia	UC Irvine (Engineering Tower)
To Whom First Disclosed	Peter Rentzepis, professor at UCI chemistry department	John Collins, postdoc at UCI
Date First Disclosed		

7. **Disclosures:**

If you have disclosed this invention to non-UC personnel (including research sponsor) then indicate when, under what circumstances, and to whom.

- a. orally Dr. Mike Krihak, Program Manager at Defense Advanced Research Projects Agency (DARPA)
- b. in writing Mike Krihak and Dr. Anantha Krishnan, also PM at DARPA. It was submitted in two proposals: one to NASA (the University Research & Engineering Institutes) and one to NSF (the Nanoscale Interdisciplinary Research Teams)
- c. by actual use, demonstration, or posters

We showed Dr. Mike Krihak the demonstration of the droplet formation under a microscope in our lab at UCI.

8. **Publication:**

Has this subject matter been published or disclosed anywhere in the form of a report (including sponsor), abstract, paper, thesis, or conference presentation? If so, where and when? Do you plan to submit a manuscript, and if so, has a manuscript been prepared? If yes, give details, including the actual or planned date of submission. If a manuscript has been accepted, give the anticipated publication date. Append a copy of the latest draft manuscript available. (See instructions for the effect of publication prior to the filing of a patent application.)

As mentioned above, we have submitted two proposals with portions of the invention. We are planning on submitting the invention to two conferences, one due December 6<sup>th</sup>, 2002 (Transducers 2003) and one due December 15<sup>th</sup>, 2002 (ASME Bioengineering Summer Conference).

9. **Prior Art:**

State all known prior art, published or unpublished, including related UC Irvine work, which bears on the invention. In the case of chemical compounds, present closely related structures? How does the invention differ from the prior art? Has a literature or patent search of this matter been made by you or for you? If so, attach copies of the most pertinent references. If none, state why not?

——No Known Prior Art

——No Prior Art Search Done

10. **Problem Solved:**

How was the problem solved in the past? What was the disadvantage to overcome?

Liposomes preparation methods and droplet formation processes have been taking place for decades. For size control, the only feasible method that is comparable is by extrusion through microporous membranes but the sizes are fixed with the pores sizes. In addition, the bulk mixing process did not allow for individual control of the composition to generate precise multilamellar vesicles or asymmetric vesicles on demand as our microfluidic device will enable.

11. **Advantages:**

State the advantages which the invention has over the prior ways of achieving the same purpose.

Programmable control of size and composition, possibility of controlled multi-lamellar and asymmetric vesicles, 100% encapsulation of reagents (e.g. to save precious drugs and reagents), controlled insertion of membrane proteins, and sorting of vesicles/droplets.

12. **Detailed Examples and/or Drawings**

Attach flow sheets of syntheses defining the contemplated scope, and detailed examples of how the invention is made and operates. Include drawings, graphs, figures, etc. to support inventive process. When available, include physical constants.

Figures 1&2 are described above. Figure 3 illustrates three different device designs to generate droplets. Figures 4&5 are photos illustrating the droplets being formed in the microfluidic devices. Figure 6 illustrates the concept of generating protein synthesis vesicles using our technique in a flow diagram.

13. **Regulatory Approval:**

Were any human or animal subjects used to obtain data to support this disclosure? If yes, did you get all necessary regulatory approvals?

No.

14. **Utility:**

What are the proposed uses for the invention? Give a detailed description of how to use it (dosages, formulations, therapeutic treatment of a disease, etc. as appropriate).

These amphiphilic vesicles are artificial cells with applications in drug delivery (including biomolecular nanomedicine such as DNA, peptides, proteins), combinatorial chemistry, nanoscale chemical reaction chambers, encapsulated protein synthesis, biomolecular power generation, and various biosensors (light, electrical, pH, ionic concentration).

15. **Commercial Use:**

Has this invention had any public or commercial use? If so, what? Where? When? If public or commercial use is expected in the immediate future, indicate what, where, and when. Include date of first order or sale, if applicable.

No.

16. **Potential Licensees or Research & Development Sponsors:**

List companies you believe might be interested in using, developing or marketing this invention. Would you be interested in collaborating with the potential licensee? ☒-X-Yes ☐—No

Liposome companies, protein synthesis companies, protein crystallization companies, Agilent Technologies, pharmaceutical companies, drug delivery companies, etc.



17. **Signatures, Names and addresses of persons mentioned in Question 2:**

Signature / Date	Signature / Date
Print Name Abraham Phillip Lee	Print Name Yung-Chieh Tan
Dept / ORU Biomedical Engineering	Dept / ORU Biomedical Engineering
Nationality U.S.A.	Nationality Taiwan, R.O.C.
Resident Street Address 11 Murasaki Street, Irvine, CA 92612	Resident Street Address
Campus address with zip+4 (zip code + zot code) BME Department, 204 Rockwell Engineering Center, Irvine Ca 92697-2715	Campus address with zip+4 (zip code + zot code) BME Department, 204 Rockwell Engineering Center, Irvine Ca 92697-2715
Campus Extension 4-9691	Campus Extension 4-9926

Note: If there are more persons please provide signatures, names and addresses on an additional sheet of paper.

18. **Non-UC Collaborator(s):**

For any person named above in #17, who is not employed full-time by the University of California, please identify other employers (e.g., Veterans Administration, Howard Hughes Medical Institute, USDA), the percent of salary time funded by such other employer, and the nature of the other employment (such as research, teaching or clinical duties).

19. **Technically Qualified Witnesses (Two Required)—invention disclosed to and understood by:**

Signature / Date	Signature / Date
Print Name	Print Name

Submit this form with ORIGINAL SIGNATURES directly to:

V.J. "Raj" Rajadhyaksha, Ph.D  
Associate Director  
Office of Technology Alliances  
380 University Tower  
4199 Campus Drive  
Irvine, CA 92797-7700

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Effective 1/1/82  
Revised 1/17/2003

Retention: 7 years after last patent expires or 10 years after the date of the last action whichever is later

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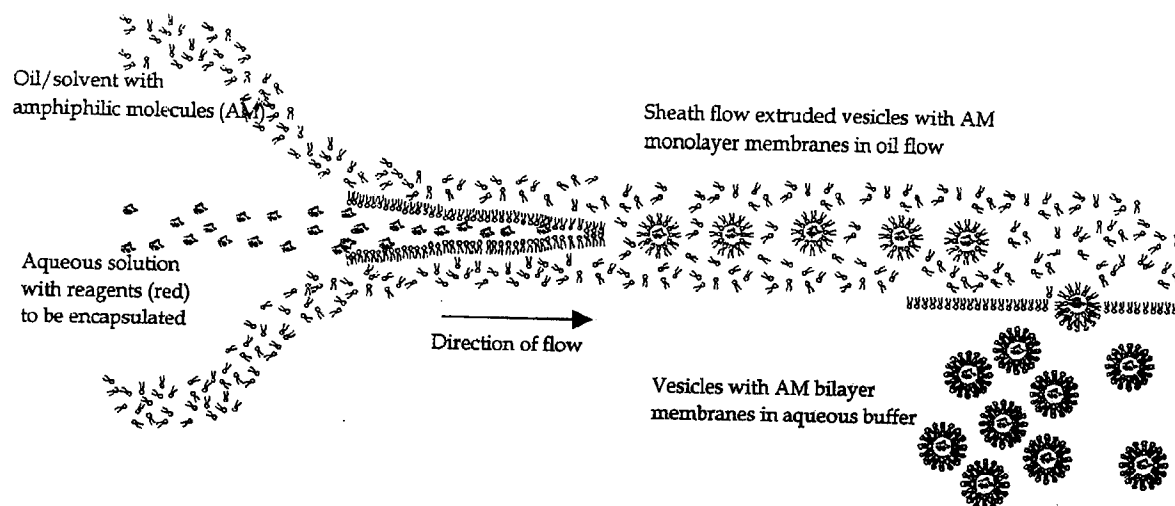


Fig. 1. General design of microfluidic device for directed self-assembly and nanomanufacturing of amphiphilic vesicles.

**Task 1.3 Integrated nanomanufacturing platforms for automation and post-assembly manipulation.** We have developed microsystem platforms that can enable the automation of complex self-assembly processes. One such platform is the magnetohydrodynamic (MHD) fluidic manifold, that can individually control the pressure/flow rates of each microchannel[46-48]. This device will be used in the 3<sup>rd</sup> year of the project when the fundamental parameters to control interfacial tension and membrane molecular concentration are determined. The MHD electrode pairs will be used for in situ characterization of the AVs such as capacitance sensors for quantifying charged molecules (e.g. DNA) in AVs[49], counting and sorting of sizes by conductivity[50] and electrical field flow fractionation [51], and for probing membrane characteristics by dielectrophoretic techniques[52].

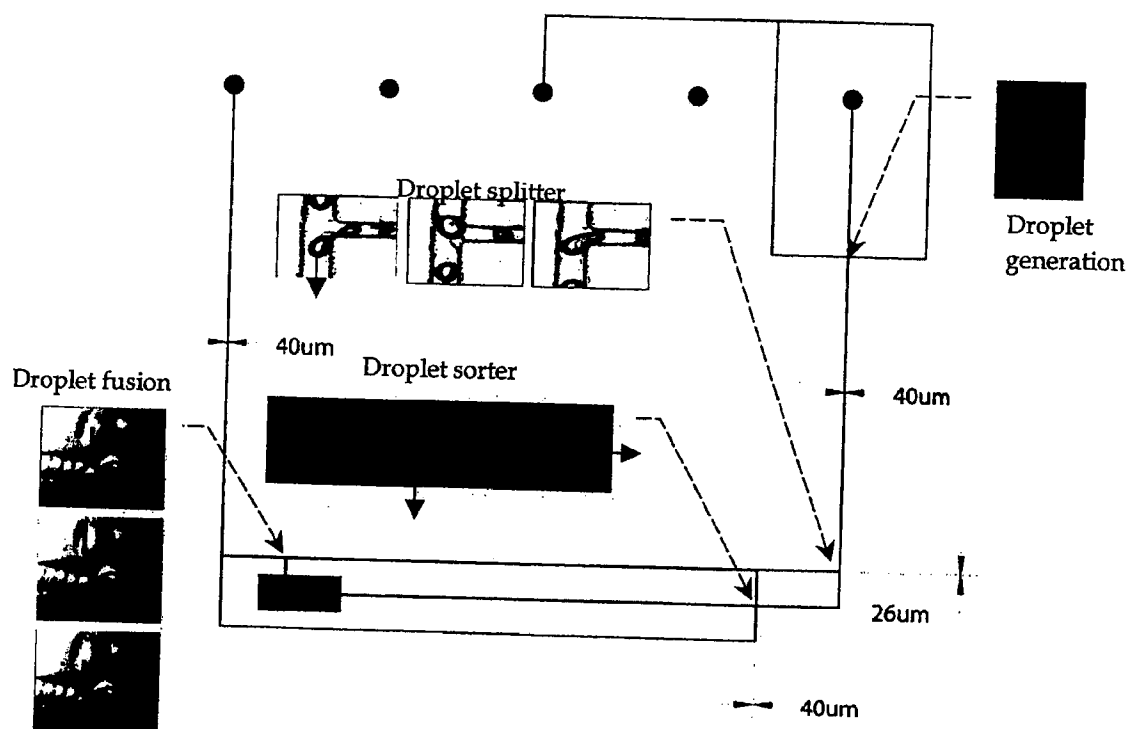


Fig. 2. Layout of an amphiphilic vesicle (AV) "nanomanufacturing" production line with major components for directed self-assembly of droplets/vesicles and post-assembly manipulation (sorting, splitting, fusing).

After the AVs are formed, it is possible to generate a complex post-assembly "nanoscale AV factory". A preliminary device layout of such a factory is depicted in Fig. 2, where in addition to AV generation, we have demonstrated droplet splitting by shearing the droplets at the corner of a microchannel bifurcation, droplet fusion by generating a large droplet as a "tapper" to force droplets to merge, and a droplet sorter that sorts according to size and density.

The primary biologically-inspired cellular functions to be implemented by the devices developed in Task 1 are described in Tasks 2 and 3. However, many other arrangements of AVs can be explored that mimic real biological cells and their networks. Our system has the potential to implement many of them. For example, a research group from Sweden [53, 54] recently developed a nanotube-vesicle network that mimics the Golgi complex in intracellular transport of protein and lipids[55]. Combinatorial synthesis using AVs can be carried out by sorting and fusing AVs with reagents and samples, providing a digital chemistry factory for synthesis and nanoassays[56]. By inserting various tags and biomarkers, it is also possible to use precisely sized AVs for particle imaging[57].

Preliminary devices were designed to nanomanufacture droplets by controlling the interfacial tension and shear gradients in between oil and water. Fig. 3 illustrates three types of designs for generating droplets, each representing a different condition and control on the interfacial tension and the shear rates. Preliminary results of oil-water microfluidic devices successfully generated water-in-oil droplets, where the sizes of the droplets ranged from  $10\text{ }\mu\text{m}$  to  $40\text{ }\mu\text{m}$  (see Fig. 4 & Fig. 5), which correspond to less than  $0.1\text{ nL}$ . The “cross-junction device” (Fig. 4) in generated droplets at 1-2 drops/sec whereas the “bowtie” device (Fig. 5) was much faster at 90 drops/sec. The cross-junction droplet generation rate can be increased in the cross-junction generator with higher flow rates. Our initial results were generated by a common flow rate syringe pump drained towards a vacuum pump with little control over the flow conditions in each microchannel. Yet we were able to roughly control the sizes and uniformity of the droplet sizes with two types of relatively simple devices. This leads us to believe that with better flow control (the current task), accurate molecular structures (Task 2), and better characterization methods (Task 3), complex features for artificial cells can be “manufactured” (Task 4). The channels were molded out of PDMS and bonded to a polystyrene substrate.

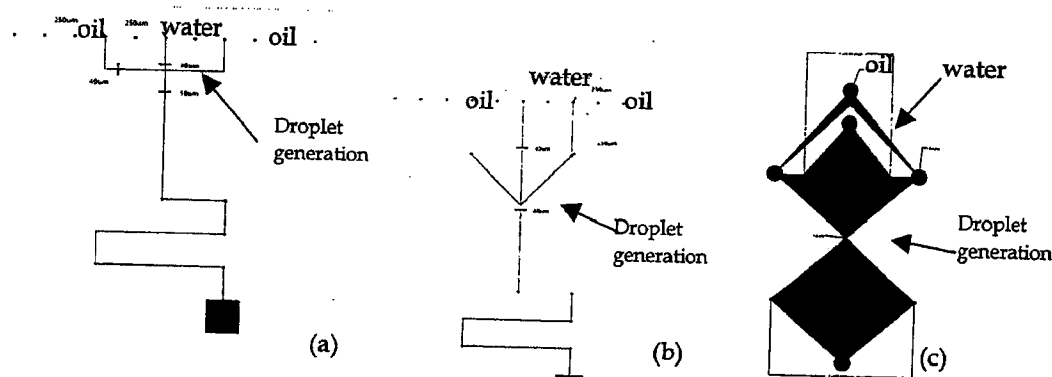


Fig. 3. Various device designs for generating amphiphilic vesicles. (a) cross-junction vesicle generator, (b) the fork section vesicle generator, (c) the bowtie vesicle generator.

Our goal will be to investigate the conditions to reliably control AV sizes ranging from  $50\text{ nm}$  to  $10\text{ }\mu\text{m}$ , the common range of organelles and cells. To date our preliminary droplets simply generate water-in-oil emulsions. For the generation of AVs, different AMs (polymers and lipids) will need to be added for membrane forming (as shown in Fig. 1). The design will have to consider the dynamic interfacial tension during the droplet forming process as the AMs aggregate towards the interfaces [12, 26, 33-35]. It is important to note that the vesicle forming process is dynamic and therefore requires accurate flow control in each channel to maintain steady state flow and uniform production of vesicles. Different flow conditions will be required to maintain steady state generation of droplets based on the dynamics of the AMs. Dynamic perturbation of flow could also enhance the control of small droplet formation by inducing Rayleigh Instability like droplet formation [36, 37]. In order to address these issues, we have acquired a piece of equipment, a microfluidic control manifold system (CAS, or Chemical Analysis Station) developed by Micronics, Inc in Redmond, WA. This will give us a LabView™ controlled fluidic station with flow rate control of 4 channels as accurate as  $0.01\text{ }\mu\text{L}$  per second. However, since their system is tailored towards industrial applications, it will be necessary to convert it to be used with our polymer chips (PDMS, polyurethane, etc.) that require much smaller channel sizes than their laser-written fluidic cards. Micronics is working with us to develop a chip-to-card interface in order to provide such a test platform (see support letter from Micronics).

Another challenge on the device development will be the microfabrication processing. It will be necessary to develop lithography for  $1\text{ }\mu\text{m}$  or less channels and free of debris, which can disrupt the flow pattern and the vesicle forming process. The design of the fluidic generation will be altered according to understanding based on the discoveries made in Task 1.2.

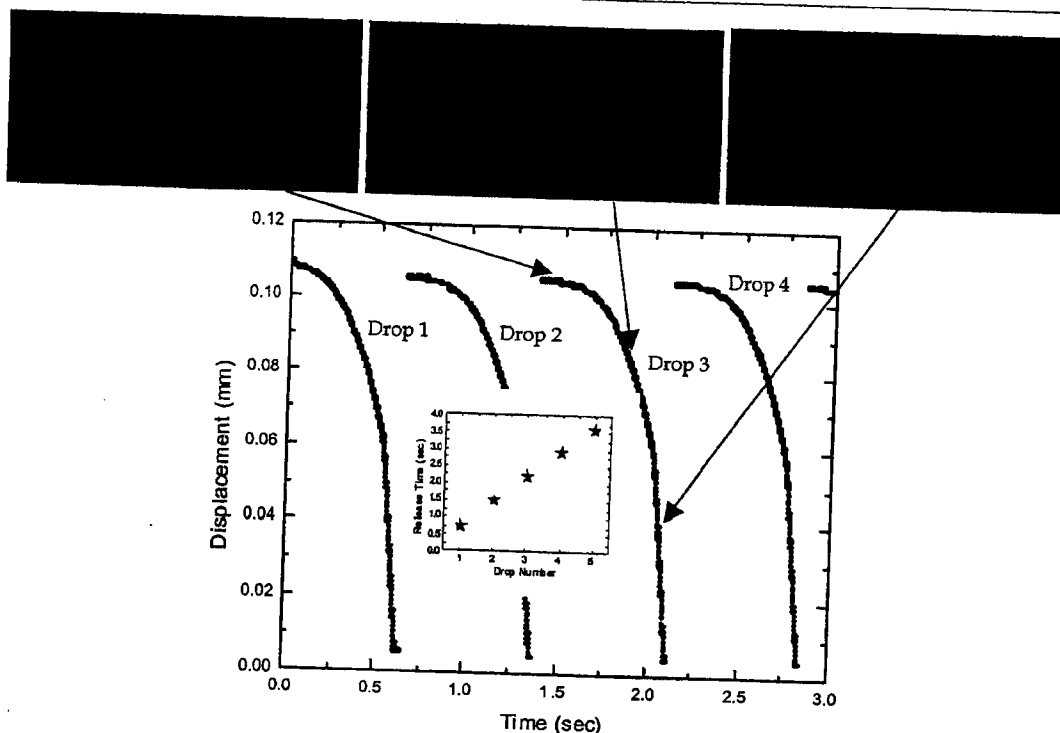


Fig. 4. Photos demonstrating the aqueous droplet formation in olive oil taken by a high speed camera. The microchannel width is  $40\mu\text{m}$ . Displacement of droplets released from the T junction is plotted at the function of time. Initially the 'seed' drop nucleates for a small time, then the drop slowly grows and finally the after the release the drops moves faster. This corresponds to the plateau region in the curve, small-sloped line and large-sloped line. The inset curve shows the number of droplets released as a function of time. The straight line implies the droplets are released at equal interval of time

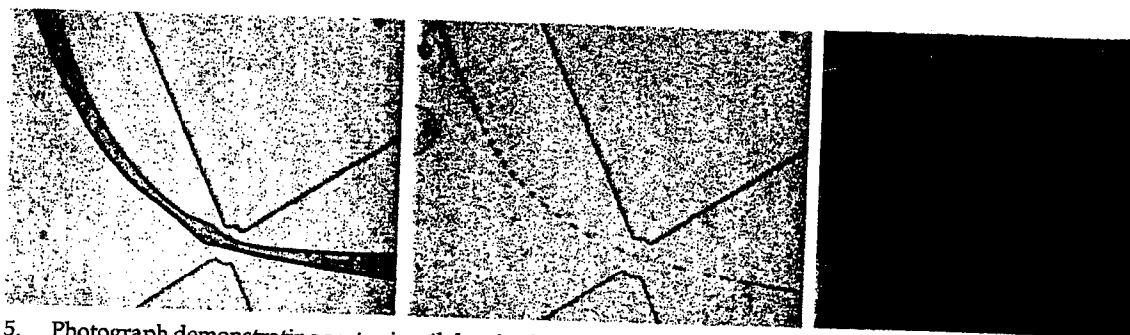


Fig. 5. Photograph demonstrating water-in-oil droplet formation in a microfluidic device. Left: a water stream is sandwiched by oil flow. As the ratio between oil and water flow rate is increased, the interfacial tension can no longer sustain a laminar flow line ([36, 40, 41]). As a result, droplets of approximately  $25\mu\text{m}$  are formed (Center). Right: Extrusion of droplets ( $150\mu\text{m}$  diameter) through the necking junction at 90 droplets per second.

Once we can consistently control the generation of AVs, more sophisticated variations of the AV nanomanufacturing device will be designed. Nano "production lines" can be set up such that one vesicle is later enwrapped by another amphiphilic layer for tailored multi-lamellar vesicles. This will be critical for the incorporating ATP synthase for "bioenergy packets" (Task 4). In addition, controlled assembly of multi-lamellar vesicles can encapsulate multiple reagents or drugs for broad applications in medicine and biology. It is also possible to generate asymmetry in the bilayers such that the inner and outer AMs are different. This is significant since biological membranes are, in fact, a fluid composition of lipids and other amphiphilic molecules [38, 39]. It will be possible to dynamically load the AVs with different

molecules, both in the membrane and encapsulated within it (e.g. membrane proteins, shear force driven controlled permeability for changing composition and/or concentration of encapsulated fluids etc.).

In combination with Task 2, where the amphiphilic polymer molecules will be grafted, the relationship between molecular structure and self-assembly parameters will be discovered. This will enable the control of vesicle shapes (spheres, rods/cylinders). Task 3 will verify the properties and efficiency of the AV nanomanufacturing device using state-of-the-art AV characterization methods.

**Task 4-2. Protein (Luciferase) synthesis in AVs.** An "artificial cell" for protein synthesis will be assembled using 1) the formulations for classical liposomes, and 2) commercially-available protein transcription/translation systems. Using the nanotechnology assembly procedure, we will encapsulate aqueous solutions containing, in particular, the complete TnT Quick Coupled Transcription/Translation system of Promega (Madison, WI). With the inclusion of luciferase control DNA designed for use with this system (also from Promega), this comprises a fully-functional transcription/translation system that produces a gene product (luciferase) that can be easily assayed using a luminometer. This complete system in aqueous solution will be encapsulated into liposomes of phosphatidylcholine and cholesterol.

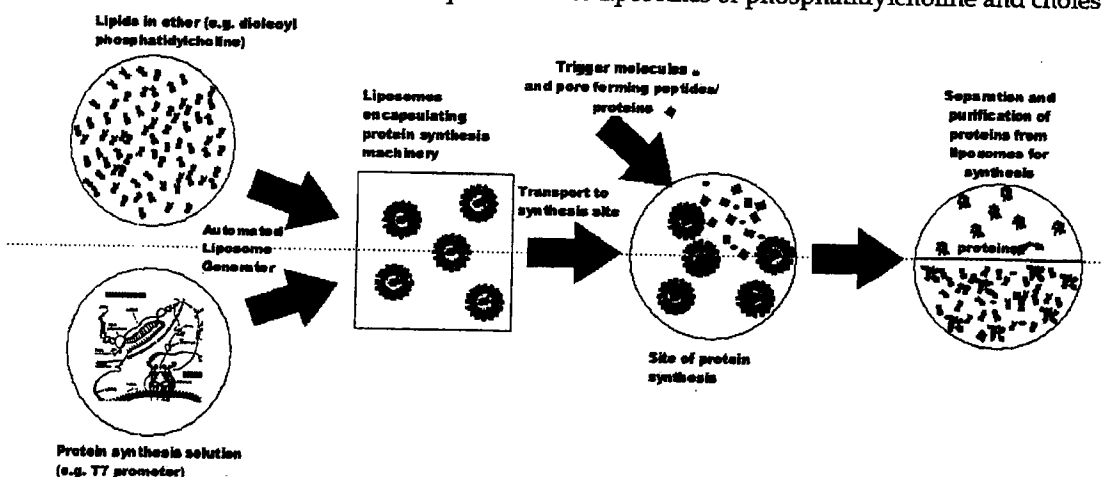


Fig. 6. Protein synthesis in liposomes.